Leading Edge
Essay
Cell

The Rational Design of an AIDS Vaccine

Daniel C. Douek,[1] Peter D. Kwong,[1] and Gary J. Nabel[1,*]
[1]Vaccine Research Center, NIAID, National Institutes of Health, Room 4502, Building 40, MSC-3005, 40 Convent Drive, Bethesda, MD 20892, USA
*Contact: gnabel@nih.gov
DOI 10.1016/j.cell.2006.02.005

The extraordinary genetic diversity and immune evasion of human immunodeficiency virus (HIV) pose significant challenges for vaccine development. AIDS vaccine design requires a scientifically driven, rational approach that encompasses the latest advances in viral molecular genetics, structural biology, and immunology.

More than 60 million people have been infected with HIV-1 since its discovery over 20 years ago, and of these, more than 20 million have died. Natural immunity to the virus is virtually nonexistent, and a vaccine to prevent AIDS remains elusive. As the toll of human suffering, social disruption, and economic instability grows, the resolution of this intractable problem remains one of the greatest opportunities to use scientific research to promote human health on a global scale. Although antiretroviral therapy has extended the lives of HIV-infected individuals, these expensive drugs are not readily available in the developing world, nor can they eradicate infection. In contrast, a successful AIDS vaccine offers the promise of preventing symptomatic disease, if not infection entirely. Arguably, more has been learned about the molecular biology, immunology, and pathogenesis of HIV-1 infection than those of any other virus in history. So why has a vaccine not emerged, and what scientific questions must be resolved to develop an effective vaccine against HIV?

The proof of concept for any successful vaccine lies in its ability to protect against infection with the pathogen. In the absence of natural immunity to the AIDS virus, scientific understanding of the disease drives an approach to vaccine development known as "rational vaccine design." From our knowledge of viral pathogenesis, hypotheses are developed about the characteristics of a successful vaccine, and these are ultimately tested through efficacy trials in humans. A key to success is to first identify the most relevant mechanisms of immune protection. For HIV, two biological features of the virus have posed the biggest impediments to developing a vaccine: its extraordinary genetic diversity (reviewed in Korber et al., 2001), and the evasive properties of its envelope protein. Precisely how HIV-1 evades the humoral (antibody) immune response is still being deciphered, but, in the estimated 70 years since HIV-1 crossed the species barrier from chimpanzees to humans (Korber et al., 2000), its contemporary diversity dwarfs that of many other entire virus families. Immune mechanisms that confer some degree of protection include the T cell response that controls disease progression (established by nonhuman primate studies), analysis of infected humans that are long-term nonprogressors, and selection of escape mutations in the epitopes of $CD8^+$ T cells. Although genetic resistance to HIV infection has been described in individuals with a mutant CCR5 chemokine receptor, an essential coreceptor for entry, this protection results from inefficient viral replication and is not mediated by immune recognition of the virus. The humoral immune response has the ability to neutralize some viruses, but mutations in the variable regions of the envelope protein continually generate viral escape variants in most infected subjects. Critical to the development of a successful AIDS vaccine will be

Table 1. Candidate AIDS Vaccines in Advanced Clinical Trials

Vector	Insert	Immune Profile	Manufacturer or Sponsor
Canary poxvirus ± protein	Env (E), Gag/Pol (B), Env (B, E)	Cellular ± humoral	Aventis/Vaxgen
rAd	Gag (B), Pol (B), Nef (B)	Cellular	Merck
DNA/rAd	Gag (B), Pol (B), Nef (B), Env (A, B, C)	Cellular ± humoral	VRC, NIAID, NIH
AAV	Gag (C), PR (C), RT (C)	Cellular	IAVI
Lipopeptides	Gag (B), Pol (B), Nef (B)	Cellular	ANRS

Description of AIDS vaccines under evaluation in phase II or phase III clinical trials. Shown are the HIV gene products (insert) delivered in the vaccine (vector), the expected immune responses (immune profile), and the manufacturer or sponsor. Letters in parentheses indicate the clade of origin for each viral gene product. rAd, replication-defective adenovirus; AAV, adeno-associated virus; Env, envelope protein; Gag, group-specific antigen; Nef, negative regulatory factor; Pol, polymerase; PR, protease; RT, reverse transcriptase.

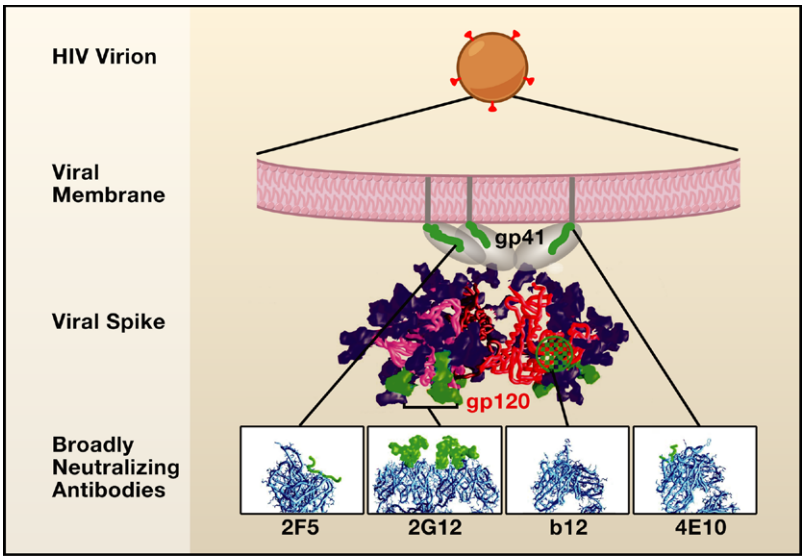

Figure 1. Structures and Targets of Neutralizing Antibodies that Block HIV Entry into Host Cells

Shown is the trimeric HIV-1 spike protein composed of three gp41 subunits (gray), three gp120 core units (light and dark red), N-linked carbohydrate (purple), and sites vulnerable to potential antibody-mediated neutralization (green). Broadly neutralizing antibodies against HIV interfere with the CD4 binding site of gp120 (antibody b12), the carbohydrate determinants of the spike (antibody 2G12), or conserved domains in the membrane-proximal region of gp120, which mediate fusion of the viral envelope with the target-cell membrane (antibodies 2F5 and 4E10) (reviewed in Burton et al., 2005). In the orientation shown, the left two gp120 molecules overlap (dark red), and a protomeric core (bright red) is clearly seen in the rightmost gp120. Note that HIV-1 gp120 is shown here in its CD4 bound conformation; the structure of unliganded SIV gp120 (Chen et al., 2005) demonstrates that considerable conformational reorganization occurs upon binding of gp120 to the CD4 receptor of host T cells. Inset figures show structures of broadly neutralizing antibodies (light blue) and, where known, their HIV-1 epitopes (green).

our ability to elicit immunoglobulins that inactivate diverse viral strains—that is, broadly neutralizing antibodies—and generate strong $CD4^+$ and $CD8^+$ T cell immune responses. Recent vaccine candidates advancing in clinical trials have focused primarily on inducing cellular immunity using gene-based vectors, such as DNA, replication-defective adenovirus, or poxvirus (Table 1). These vaccines primarily intend to stimulate either $CD4^+$ and $CD8^+$ T cell responses or $CD8^+$ T cells alone. Success in eliciting broadly neutralizing antibodies has been limited to date, although it is anticipated that such candidate vaccines will be tested in the future, either alone or in combination with T cell-based vaccines.

Generating Broadly Neutralizing Antibodies

Structural and antigenic characterization of the HIV-1 envelope reveals unprecedented mechanisms for evading the host antibody response. The viral spike is composed of three gp120-gp41 glycoproteins. It binds to CD4 and a coreceptor on the host T cell surface and promotes fusion of HIV-1 and host-cell membranes, enabling virus entry (see Figure 1). Much of its exposed surface is cloaked by N-linked glycan, which is produced by the host cellular machinery and is largely unrecognized by the immune system. This glycan surface provides an evolutionarily efficient means of escape from neutralizing antibodies; a small number of mutations can give rise to significant changes in glycan structures that confer resistance to neutralization. The virus uses other evasive mechanisms: immunodominant regions that are occluded in the native oligomeric spike protein of the virus are exposed in viral debris or in inactive forms of the spike protein. These immunodominant regions generate HIV-specific antibodies, which do not bind to the functional spike. Conformational masking also contributes to the resistance of the virus to neutralizing antibodies. The coreceptor binding site on gp120 of HIV is highly conserved, and neutralizing antibodies develop readily against it. However, on functional viral spikes, the potentially susceptible site of coreceptor binding is formed only after attachment of gp120 to CD4 on the host-cell surface, preventing access of neutralizing antibodies to an otherwise highly conserved binding site. When these mechanisms of humoral evasion are coupled to the extraordinary natural diversity of the virus, the task of generating high titers of broadly reactive, neutralizing antibody in vaccine subjects is daunting.

Fortuitously, the technologies that reveal the challenges of eliciting such antibodies provide insights into potential vulnerabilities. Monoclonal antibody and phage display analyses have identified a few broadly neutralizing antibodies. For example, antibodies such as 2F5, 4E10, 2G12, and b12 neutralize a significant percentage of circulating HIV-1 primary isolates (Burton et al., 2005), and their molecular structures and targets are now well characterized (Figure 1). Why are these antibodies effective?

One answer may be that they recognize functionally constrained, conserved, and exposed structures—that is, the viral spike must find a receptor and then fuse viral and target-cell membranes. These twin functions of "finding" and "fusing" provide constraints on the viral spike, which may be recognized by such antibodies as b12 (CD4 binding) or 2F5 and 4E10 (membrane fusion). The functional rationale for conservation of the 2G12 carbohydrate epitope, which is largely limited to clade B viruses, is less clear and may relate to preserving advantageous interactions with the innate immune system (for example, interaction with the carbohydrate binding receptor DC-SIGN) or constraints on carbohydrate density related to glycan shielding.

The information derived from structural analysis of broadly neutralizing

monoclonal antibodies informs vaccine design. Precise characterization of the structures recognized by these antibodies is the first step in creating polypeptides or small molecules that mimic such epitopes. To this end, significant effort has been made to gain an atomic-level understanding of susceptible epitopes and their interaction with neutralizing antibodies. The guiding hypothesis is that the proper presentation of a functionally conserved, susceptible epitope will lead to the elicitation of antibodies that recognize the target epitope and neutralize the virus. To overcome the conformational flexibility of the HIV envelope protein, modern tools of protein design can be used to create mutations that fix gp120 into the form recognized by the CD4 receptor or by broadly neutralizing antibodies. To help focus the immune response, one can remove immunodominant regions, thus paring the envelope to critically conserved regions of the core or outer domain, or one can mask immunodominant regions with carbohydrate to make them immunologically silent. Another strategy regarding epitope presentation involves the creation of epitope-transplant scaffolds. In this scaffolding strategy, the target epitope is transplanted into a foreign scaffold that replicates both the conformation and the surface accessibility of the epitope as recognized by a broadly neutralizing antibody. These approaches apply structural information to vaccine design: Although attractive, this process remains a working model and has yet to achieve the goal of solving the neutralizing-antibody problem.

Whether immunogens created by epitope mimicry will allow antibodies to be elicited with properties similar to the original broadly neutralizing antibody will depend on a number of variables: the uniqueness of the template antibody, the degree of structural mimicry between epitope mimetic and antibody bound epitope, and the ability of the humoral immune system to recreate specific immune responses. The tools of conformational stabilization, epitope focusing, and scaffold transplantation have much to contribute to rational vaccine design. Once the appropriate antibody immunogens are generated, it may be equally important to ensure that relevant antibodies are synthesized not only in the systemic circulation but also at mucosal sites that serve as portals of primary infection.

The Cellular Immune Response

In the past, "traditional" vaccine design has tended toward an empirical approach, which has often been at odds with traditional cellular immunology—a discipline that is firmly hypothesis driven and centered on well-characterized inbred mice. Human immunology, particularly in the field of infectious disease, has often relied on phenomenological descriptions of disease with imprecise measures of immune responsiveness. However, the rational approach to vaccine design for HIV has necessitated a revised approach to the study of cellular immunity in humans. Fortunately, recent technological advances have furnished enormous analytical power that has facilitated an understanding of the mechanisms of immune protection to infection.

Studies of HIV pathogenesis indicate that the bulk of $CD4^+$ T cell depletion occurs on a massive scale, predominantly at intestinal mucosal surfaces, within a short time period after the onset of infection. These findings were made possible through the ability to sample multiple lymphoid tissues in infected nonhuman primates and humans and to perform sophisticated measurements of numerous immunological parameters. These findings have fundamental implications regarding the site and timing of the delivery of therapeutic agents to HIV-exposed individuals and also affirm that the abatement of HIV replication at mucosal surfaces is likely to be integral to the success of a prophylactic vaccine.

There is strong evidence from human studies that virus-specific T cell responses are critical to the control of viral replication in many chronic persistent infections, including those caused by cytomegalovirus (CMV), Epstein-Barr virus (EBV), and hepatitis viruses B and C. However, for HIV, the questions remain whether we can (1) define the mechanisms and correlates of immunity and (2) translate those principles into the design of effective vaccines. Critical to the success of a T cell-based vaccine are five major features of the HIV-specific cellular immune response: its size, phenotype, function, structure, and anatomical location.

An effective $CD8^+$ T cell response is likely to be required for control of HIV replication in the chronic phase of the disease. In the case of chimeric simian and human immunodeficiency viruses (SHIV) and perhaps simian immunodeficiency viruses (SIV), vaccine-induced T cell responses are associated with an improved outcome. However, the quantitative frequency alone of either $CD4^+$ or $CD8^+$ virus-specific T cells seems not to correlate with viral load or clinical outcome, implying that quality rather than quantity contributes to an effective immune response. Definition of the character of such immune responses requires a refined and comprehensive approach to immune analysis in humans. Indeed, the recent use of polychromatic flow cytometry to measure multiple phenotypic and functional parameters of T cells stimulated with vast libraries of peptides that represent the entire HIV proteome has revealed valuable immune correlates. Individuals with nonprogressive chronic HIV disease possess HIV-specific T cells whose phenotype and elaboration of multiple effector functions more closely resemble those of CMV- and EBV-specific T cells, which apparently successfully contain their respective infections (Pantaleo and Koup, 2004). An inherent problem with an observational approach, however, is one of causality, such that the phenotype and functional profile of virus-specific T cells may be a consequence of virus control rather than its cause. Nevertheless, it is highly likely that, with the extension of such multiparameter immune analysis to large-scale vaccine clinical trials, we will begin to establish rules that determine the correlates of effective immunity elicited by vaccination.

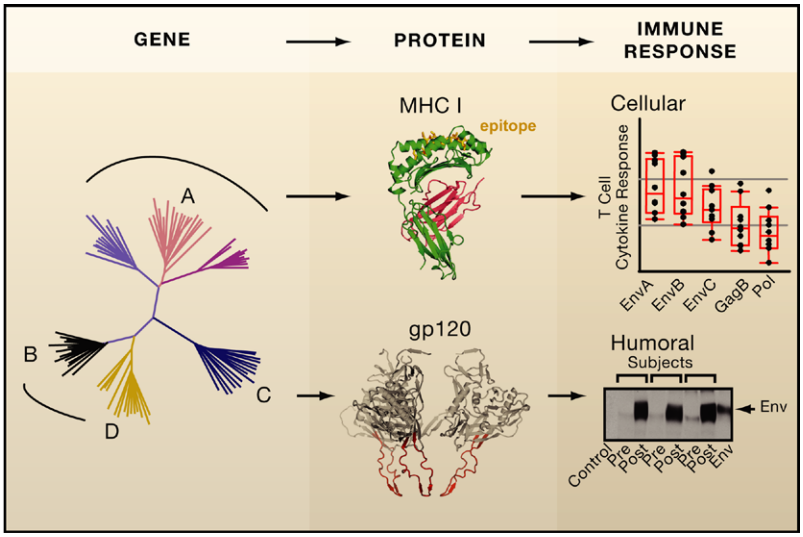

Figure 2. Rational Vaccine Design
Improving our understanding of vaccine immunogenicity and subsequent disease protection relies on applying knowledge about (1) viral genetic diversity (depicted in the dendrogram), (2) the protein structure of major histocompatibility complex I (MHC I) proteins of the host and gp120 of HIV-1, and (3) the ability of these proteins to generate protective immunity. Improved mechanistic understanding of these three areas, as well as of vectors, adjuvants, and modes of delivery, will accelerate future vaccine development. The ultimate aim of rational vaccine design is to develop scientific principles and algorithms that predict protective immune responses in the host based on knowledge of the virus and the host immune system. This process is likely to be iterative and to provide information about immune epitopes that will guide the selection of the vectors and gene products most likely to confer immune protection against a specific infectious agent.

The "structure" of a virus-specific T cell response comprises a complex set of features including the number of epitopes targeted (response breadth) and their relative immunodominance, the clonal composition of epitope-specific T cell responses, and the mode of interaction between epitope-specific T cell receptors and their cognate antigens. Importantly, this line of investigation relates directly to the phenomenon of viral epitope escape by sequence variation, perhaps one of the greatest obstacles to effective control of HIV replication. Evidence is accumulating that the preferential targeting of particular HIV epitopes by the $CD8^+$ T cell response may confer better, or sometimes worse, disease outcomes. These observations are reflected in the well-described associations of HLA histocompatibility complex variants with disease outcome in humans and likely reflect the balance between the host immune response to a viral epitope, the propensity of the epitope to escape the immune response, and the biological fitness of viruses to contain such sequence variation. There is also evidence that particular T cell clones may directly affect patterns of epitope escape. The key is that the accumulation of data from many thousands of HIV-infected individuals worldwide that identify preferentially targeted epitopes, the timing and nature of epitope escape, epitope-specific T cell clonal usage, and HLA type and disease outcome should allow us to predict the nature of the T cell response that we need to elicit with a vaccine to prevent disease (or, in certain cases, the type of T cell response that should be avoided in particular human populations). Furthermore, the principles established could then be applied to the rational design of immunogens that elicit desired T cell responses and can be incorporated in a successful vaccine.

In truth, there is still no direct evidence that HIV-specific T cell responses prevent or retard HIV-1 disease progression. Yet what we have learned from the phenotypic, functional, and structural analysis of virus-specific cellular responses to CMV, EBV, SIV, HIV, and other viral infections suggests principles for an effective vaccine. Such a vaccine against HIV should elicit (1) a high frequency of polyfunctional T cells, especially those with the ability to secrete interleukin-2 and to proliferate; (2) T cells whose antigen receptors show high functional avidity for viral epitopes; (3) T cells that target epitopes where viral fitness constraints curb their ability to mutate; (4) T cells with recognition properties that are better able to detect escape mutations and viral quasispecies; and (5) T cells at mucosal surfaces, which are the first T cells likely to come into contact with HIV-infected cells. All of these principles will guide critical aspects of vaccine design, including the route of immunization, mode of antigen presentation, antigen dose and persistence, and use of immunomodulatory adjuvants.

The Future: A Global and Scientific Perspective

The majority of HIV-infected individuals do not live in nations with immediate access to the advanced technologies required for sophisticated immune analyses. Clinical trials therefore require unprecedented cooperation between scientists in the developed and the developing world. This effort will require the cooperation of governments internationally, nongovernmental organizations, and the private sector and the development of clinical and laboratory infrastructure. Clinical trials must be performed with appropriate ethical review, together with the willingness of all partners to evaluate vaccine efficacy rigorously and with transparency. Thus, we must further develop these technologies in such a way that they may be used in the field as part of the large clinical trials that will surely point the way toward an effective HIV vaccine. These goals have been embraced in the research community through such efforts as the HIV Global Enterprise, an international consortium of collaborative nongovernmental and governmental organizations.

It is important to recognize that there are several potential outcomes

in such trials. One possibility is that the vaccine will prevent infection. This response, typical of classical vaccines, is unlikely to occur with first-generation prototypes of AIDS vaccines. More likely, the vaccine may affect the clinical course of the disease, not preventing infection but instead reducing the viral load and prolonging symptom-free survival. Finally, such a vaccine may or may not affect person-to-person transmission, a parameter that will require independent evaluation and that will be essential for halting the spread of AIDS. These efforts will benefit from the definition of immune correlates and the use of molecular genetic analyses to accelerate clinical evaluation. Without such innovations, it would probably take decades more to develop a highly effective vaccine.

In the future, knowledge gained from clinical efficacy studies of HIV vaccines should accelerate the development of improved vaccines. At the same time, such trials will advance our understanding of new immunization vectors not only for AIDS but also for emerging infectious diseases. Critical for progress will be expanded knowledge of protein structure, antigenicity and immunogenicity, vector development, and the correlates of protective immunity. Although development of an effective vaccine against HIV-1 must overcome enormous barriers, the tripartite task of deciphering viral genetic diversity, manipulating envelope protein structure and MHC I epitope recognition, and inducing protective immunity provides a rational system with which to tackle the problem of the ability of HIV to evade neutralizing antibodies and cytotoxic T cells (Figure 2). Application of this knowledge through bioinformatics, systems biology, bench science, and clinical trials will lead to improved paradigms for vaccine design and will facilitate the identification of effective preventive vaccines in the future.

ACKNOWLEDGMENTS

We thank Ati Tislerics for assistance with manuscript preparation; Brenda Hartman, Chih-chin Huang, and Toni Miller for figure preparation; and Dennis Burton, John Mascola, Larry Corey, Richard Koup, Bart Haynes, Peggy Johnston, and Barney Graham for comments on the manuscript. This research was supported by the Intramural Research Program of the US National Institutes of Health, Vaccine Research Center, NIAID.

REFERENCES

Burton, D.R., Stanfield, R.L., and Wilson, I.A. (2005). Proc. Natl. Acad. Sci. USA *102*, 14943–14948.

Chen, B., Vogan, E.M., Gong, H., Skehel, J.J., Wiley, D.C., and Harrison, S.C. (2005). Nature *433*, 834–841.

Korber, B., Muldoon, M., Theiler, J., Gao, F., Gupta, R., Lapedes, A., Hahn, B.H., Wolinsky, S., and Bhattacharya, T. (2000). Science *288*, 1789–1796.

Korber, B., Gaschen, B., Yusim, K., Thakallapally, R., Kesmir, C., and Detours, V. (2001). Br. Med. Bull. *58*, 19–42.

Pantaleo, G., and Koup, R.A. (2004). Nat. Med. *10*, 806–810.